modified in order to have a composition of at least 15.00-35.00 mole % lysine; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.

99. (new) A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and modified in order to have a composition of at least 15.00-25.00 mole % lysine; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.

REMARKS

Claims 9, 12, 14, 18, 19, 21, 28, 29, 30, 31, 59, 61, 63, 64, 65, 69, 70, 72, 76, 78, 79, 80, 81, 82, 83, 84 and 87 have been amended. New claims 98 and 99 have been added. Support for the amendments and new claims can be found in the specification, particularly the pages as noted herein.

Attached hereto as **Appendix D** is a marked version of the claims as amended by the current amendment. The attached pages are captioned "Marked Version of Claims."

The basis for these amendments is set forth below.

COMPLIANCE WITH THE SEQUENCE RULES

5. Applicants have amended pages 49-54 of the specification to refer to Seq. ID No. where appropriate. The Seq. ID Nos. added correspond with the Seq. ID Nos. submitted with the Amendment and Response dated November 28, 2001. The chart below shows the corresponding Seq. ID No. for each oligonucleotide sequence.

Oligonucleotide Sequence	Seq. ID No.
N4394	54
N4395	55
N4396	56
N4397	57

N5045	58
N5046	59
N13561	60
N13562	61
N13563	62
N13564	63
N13565	64
N13905	65
N14471	66
N14472	67
N13771	68
N22098	69
N22099	70
N23923	71
N23924	72
N26671	73
N26672	74

Objections to the Specification

8. The Examiner has objected to the Abstract for not completely describing the disclosed subject matter. The Examiner suggested that genus/species of the organisms from which the initial polypeptides of the invention were obtained are required. Applicants thank the Examiner for this suggestion and have amended the Abstract accordingly. Applicants submit that this objection has now been overcome.

Rejections to the Claims

9. The Examiner has objected to the amendments to the claims under 35 U.S.C. 132 on the basis that they introduce new matter into the disclosure. The Examiner has maintained two points related to new matter.

9. a

The Examiner objects to the language in claim 59, "at least 60% sequence identity..... Seq. ID No.: 2". Applicants pointed out in the previous response that polypeptide variants are encompassed within the inventions, for example on P. 26, line 19, and that polypeptide variants are defined on page 12, lines 1-3 as at least about 55%, 60%, 70%, 80%, or preferably at least about 90% and more preferably at least about 95% sequence identity to the modified protein. The Examiner maintained the new matter rejection on the basis that the Examiner finds no definition of "the modified protein" being exactly Seq. ID No. 2.

Applicants respectfully request reconsideration of this new matter rejection. The focus of the specification is on the modification of Seq. ID No.2. For example, on page 26 lines 6-9, the specification states that 'Proteins of the present invention include proteins derived from the native protein by deletion (so called truncation), addition, or substitution of one or more amino acids at one or more sites in the native protein." Starting on page 39, line 1, the modification of wild-type CI-2 is discussed. In addition, there are many other parts of the specification that discuss the modification of a CI-2 protein, such as the definitions of a "CI-2 derived" polypeptide and a "CI-2 like" polypeptide on page 8, lines 13-31. Applicants submit that one of ordinary skill in the art would understand, in the context of the specification, that the term modified proteins encompass the modified CI-2 proteins taught in the specification. Applicants submit that this objection has now been overcome.

9. b

The Examiner objects to the language in claims 61, 72, 74 and 76 to Seq. ID No. 2, positions 19-83, 19-53 and 63-83. Applicants respectfully traverse this objection.

With respect to claims 72 and 76, claiming in reference to positions 19-83, Applicants teach, on page 40, line 20, that the first 18 residues in the wild type CI-2 (Seq. ID No. 2) do not assume any ordered conformation and may be truncated. Similarly, Applicants teach on page 43, line 31, to page 44, line 1, that in one embodiment "the truncated version excludes the region corresponding to the amino terminal 17 or 18 amino acids of Seq. ID No. 2." Figure 1 is a clear indication that Applicants intended, and in fact made, embodiments in which the modifications were made to positions 19-83 of Seq. ID No. 2. However, to clarify that such embodiments are not new matter, Applicants have amended these claims so that Seq. ID No. 4 (which encompasses positions 19-83) is used as the wild type reference sequence. The format of Figure 1 clearly shows the emphasis in making the modifications in these positions of the wild type CI-2 protein, and as pointed out on page 39, lines 6-8, of the specification, "A truncated form of wild type CI-2 used in the present study (Seq. ID. No. 4) comprises residues 19 through 83 of the full length wild-type plus a start methionine." Applicants submit that claims 72 and 76, as amended, do not contain new matter and the objection should be withdrawn.

With respect to claims 61, the Applicants point out that the portion of the claim that refers to positions 19-53 and 70-83 have been cancelled. Claim 74 has also been cancelled. Accordingly, the new matter rejection has been rendered moot.

Applicants also note that due to the amendments of Claim 61, the dependency of claims 63-65 and 69 and 70 has been changed to Claim 72. Should these claims be deemed allowable, Applicants will cancel and renumber the claims so that they do not depend from a later claim.

Applicants further point out that Examiner required that the Applicant's point out precisely (page and line number) where in the instant application...clear support for the amendments can be found." Applicants have pointed these page and line numbers out above, and submit that one of ordinary skill in the art, looking at the specification, would understand that the inventor taught that which was claimed. In the case of Vas-Cath Inc. v. Mahurkar, 19 USPQ2d 1119, the Federal Circuit cited Ralston Purina, 227 USPQ 179, for the proposition that

"ranges found in applicant's claims need not correspond *exactly* to those disclosed in parent application; issue is whether one skilled in the art could derive the claimed ranges from parent's disclosure."

Claim Rejections – 35 USC Section 112, second paragraph

38. The Examiner has maintained the rejection of claims 29 and 62 as indefinite, for the use of the term "conservatively substituted variants". The Examiner notes that the specification, on page 10, refers to both "conservative substitutions" and "conservative and essential substitutions". Applicants intended that this term refer to the "conservative substitutions" as defined in the specification and as listed in the previous office action response mailed May 3, 2002. To further clarify, Applicants have amended claims 29 to refer specifically to the term "conservative substitution." Claim 62 has been deleted. Applicants submit that this rejection has been overcome.

Claim Rejections – 35 U.S.C. 112, first paragraph

42. The Examiner has maintained the rejection of Claims 29, 30, 57 and 62 under 35 U.S.C. 112, first paragraph. The Examiner suggests the inclusion of a broad structural limitation, such as 30% identity to Seq. ID No. 2. Applicants have added the suggested limitation to claims 29 and 30. This limitation has been added to claim 57 by virtue of its dependency on claim 30. Claim 62 has been deleted. Applicants submit that this rejection has been overcome.

43. The Examiner rejects claim 32 under 35 U.S.C. 112, first paragraph. Claim 32 has been deleted.

44. The Examiner rejects claim 87 under 35 U.S.C. 112, first paragraph. The Examiner refers to the discussion regarding the rejection of claims 29, 30, 57 and 62. In that discussion the Examiner suggests the inclusion of a broad structural

limitation, such as 30% identity to Seq. ID No. 2. Applicants have added the suggested limitation to claim 87. Applicants submit that this rejection has been overcome.

Claim Rejections – 35 U.S.C. 102

47. The Examiner has maintained the previous rejection of claim 9 under 35 U.S.C. 102. The Examiner has not yet considered Applicants arguments concerning the priority date granted to claim 9 since the file for 08/740,682 was not available. Applicants point out that 08/740,682 was abandoned and the file should be available.

Klebber Janske is cited as prior art because it contains 10 lysine residues (14.7 mole % lysine). Applicants have amended claim 9 to delete the reference to 15-35 mole % lysine so it can not be suggested that claim 9 reads on the Klebber Janske sequence.

Applicants have added new claim 98, which is the same as claim 9 except that new claim 98 refers to only the lysine range. Applicants submit that 14.7 mole % lysine is not within the range of 15-35 mole % lysine. To clarify, Applicants have amended claim 98 to specify the range as 15.00 – 35.00 mole % lysine. Applicants have also added a dependent claim 99 to a range of 15.00 – 25.00% mole % lysine. Applicants note that 08/740,682 teaches 13 positions (15.66 mole %) for lysine substitution, Seq. ID. No. 4 (of 08/740,682) with a mole % lysine of 21.6%, and a full length engineered CI-2 containing 21 lysine residues (25.3 mole %) which was expressed in and purified from e-coli (08/740,682, page 8, lines 12-14).

48. The Examiner has maintained the rejection of claims 19 and 21 under 35 U.S.C. 102(b) as being anticipated by Cordero et. al. The Examiner notes that if claims 19-21 are amended to read ---A polypeptide comprising Seq. ID. No. 2 modified to contain two or more modifications...---- , the instant rejection would be obviated. Applicants have amended claims 19 and 21 accordingly and thank

the Examiner for the suggestion.

Objections to the Specification

49. The amendment was objected to under 35 U.S.C. 132 for added matter.

49(a). Claim 62 has been deleted, thus obviating the rejection.

49(b) to 49(h). The Examiner has objected to claims 78 -84 on the basis that the claimed percentages introduce new matter. While the Applicants do not believe this to be the case for the reasons cited in 9e above, Applicants have amended claims 78, 79, 81 and 83 to refer to 80% sequence identity, and claims 80, 82 and 84 to refer to 90% sequence identity. Claim 78 has been amended to refer to the full length wild type CI-2 in Seq. ID No. 2. Exact support for these ranges is found on page 5, lines 24-25, of PCT/US97/20441, which states that "In one embodiment, the polypeptide exhibits 80% identity and in other embodiment, 90%." The BHL 1, 2, 3, 3N, 1N, 2N, and Wild Type CI-2 (83aa) were disclosed in PCT/US97/20441 as Seq. ID Nos. 1 and 2 for BHL 1; Seq. ID Nos. 3 and 4 for BHL 2; Seq. ID Nos. 5 and 6 for BHL 3; Seq. ID Nos. 7 and 8 for BHL 3N; Seq. ID Nos. 9 and 10 for BHL 1N; Seq. ID Nos. 11 and 12 for BHL 2N; and Seq. ID Nos. 13 and 14 for Wild Type CI-2 (83aa). The present application, serial No. 09/311, 689, lists Wild Type CI-2 as Seq. ID No. 2; BHL 1 as Seq. ID No. 6; BHL 2 as Seq. ID No. 8; and BHL 3 as Seq. ID No. 10. Further, Applicants note that PCT/US97/20441, page 91, lines 10 –14, discloses stabilization of the modified sequences through the use of non-native disulfide bonds.

49 (i). The Examiner has objected to claim 87, stating that "no reference to the species of SEQ ID Nos: ....35-53 containing non-native disulfide bonds is cited." Applicants have deleted Seq. ID Nos. 24, 26, 28, 30 and 32 from the claim.

Applicants point to page 8, line 16-31 of the specfication, where it states that "A 'CI-2 like' polypeptide refers to...a polypeptide with at least 30% amino acid sequence identity with corresponding region of Seq. ID Nos. 2 or 4 or 20; or a CI-2-like polypeptide with modifications identified in CI-2; or a protease inhibitor with an active site loop typically betwee 53 and 70; or a CI-2 homologue modified to enhance its nutritional value by altering the amino acid residues at positions corresponding to those defined herein." The definition goes on to state the homologues listed as Seq. ID Nos. 35-53. These sequences are also identified as CI-2 like sequences on page 5, lines 18 – 38 of the specification. It is clear that the Applicants taught that the CI-2 homologues identified in Seq. ID Nos. 35 – 53 may be modified in the same manner as wild type CI –2 to function in the present invention. This includes the addition of non-native disulfide bonds to the CI-2 like polypeptide sequences of Seq. ID Nos. 35-53. Applicants respectfully request reconsideration of this objection.

Claim Objections

50. Claim 62 has been deleted, thus obviating its objection under 37 C.F.R. 1.75 (c).

Claim Rejections – 35 U.S.C. 112

51. Claim 12 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The Examiner states that "The phrase 'wild type CI-2' is unclear since, in Figure 1, both Seq. ID Nos. 2 and 4 are described as wild-type. Applicants note that Seq. ID No. 4 represents the non-truncated portion of Seq. ID No. 2, and both sequences are in fact wild type. To avoid confusion Applicants have amended claim 12 to refer to Seq. ID No. 4 rather than wild type CI-2.

52. Claim 14 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The Examiner states that the transitional phrase should be ---further comprising --- and not "comprising." Applicants have amended this claim

accordingly and thank the Examiner for this suggestion.

53. Claim 18 was rejected under 35 U.S.C. 112, second paragraph, as being indefinite. The Examiner states that the word "about" is unclear in reference to about eighteen additional residues and that its metes and bounds is confusing. Applicants have amended claim 18 to delete the word "about," thus obviating this rejection.

54. Claim 32 has been deleted, thus obviating the rejection under 35 U.S.C. 112.

55. Claims 59, 61, 72, 74, 76, 78-84 and 87 and their dependent claims (Claims 60, 62-71, 73, 75, 77, 85 and 86) were rejected under 35 U.S.C. 112, first paragraph, written description – new matter, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants respectfully disagree and request reconsideration of this rejection.

The focus of the specification is on the modification of wild type CI-2. For example, on page 26 lines 6-9, the specification states that 'Proteins of the present invention include proteins derived from the native protein by deletion (so called truncation), addition, or substitution of one or more amino acids at one or more sites in the native protein." Starting on page 39, line 1, the modification of wild-type CI-2 is discussed. In addition, there are many other parts of the specification that discuss the modification of a CI-2 protein to increase its nutritional value, such as the definitions of a "CI-2 derived" polypeptide and a "CI-2 like" polypeptide on page lines 13-31 of page 8. Polypeptide variants are encompassed within the invention, for example on page 26, line 5, to page 27, lines 10, and those polypeptide variants are specifically defined on page 12, lines 1-3 as at least about 55%, 60%, 70%, 80%, or preferably at least about 90% and more preferably at least about 95% sequence identity to the modified protein. One of ordinary skill in the art would understand, in the context of the

specification, that the term modified proteins encompass the modification to Seq. ID Nos. 2 and 4 as taught in the specification.

Applicants further note that the language objected to has been deleted from claim 61, and that claims 74 and 75 have been deleted. Claims 78 – 84 and 87 have been amended as noted in 49 above.

In light of the foregoing arguments and amendments, Applicants submit that these rejections have been overcome.

Claim Rejections – 35 U.S.C. 101

56. Claims 29-32 and 58-59 were rejected under 35 U.S.C. 101. The Examiner suggests inserting the term "isolated" before the claimed product in the preamble. Claims 29-31and 59 have been so amended (and claims 57 and 58 by virtue of their dependency). Claim 32 has been deleted. Applicants thank the Examiner for this suggestion.

Claim Rejections – 35 U.S.C. 102

57. Claim 28 was rejected under 35 U.S.C. 102(b) as being anticipated by Cordero et. al. The Examiner notes that if Claim 28 was amended to read --- A polypeptide comprising Seq. ID No. 2 modified to contain three or more modifications...--- , the instant rejection would be obviated. Claim 28 has been so amended and the Applicants thank the Examiner for this suggestion.

58. Claim 59 was rejected under 35 U.S.C. 102(b) as being anticipated by Williamson et al. Applicants have cancelled claim 60 and have incorporated the more than ten lysine limitation of claim 60 into claim 59.

CONCLUSION

Applicants respectfully submit that in light of the foregoing amendments and remarks, pending claims 9-25, 28-31, 54-59, 61, 63-67, 69-74, 76-87 and 96-99 are in condition for allowance. Further examination, reconsideration, and allowance of the claims are respectfully requested. If prosecution toward allowance could be furthered by a telephone call to the undersigned attorney for Applicants (515-254-2823), one is earnestly requested.

Respectfully submitted,
Rao, *et al.*

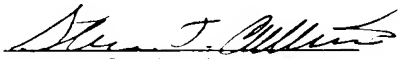
By 
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Exhibit A

Amendments to Specification, Pages 49 – 54, Marked

(i.e., substrates, products, inhibitors, activators), pH, ionic strength, and temperature will be controlled so as to obtain useful kinetic data and determine the presence of absence of a compound that binds or modulates protease inhibitor polypeptide activity. Methods of measuring enzyme kinetics is well known in the art. See, e.g., Segel, *Biochemical Calculations*, 2nd ed., John Wiley and Sons, New York (1976).

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Example 1- Construction of Expression Cassettes

Vector construction was based upon the published WT CI-2A sequence information Williamson *et al,* Eur. J. Biochem 165: 99-106 (1987) and SEQ ID NO 1. Methods for obtaining full length or truncated wild-type CI-2 DNA include, but are not limited to PCR amplification, from a barley (or other plant) endosperm cDNA library using oligonucleotides derived from Seq. ID No 1 or from the published sequence *supra,* using probes derived from the same on a barley endosperm cDNA library, or using a set of overlapping oligonucleotides that encompass the gene, or having the gene synthesized by a commercial vendor such as The Midland Certified Regeant Company (Midland, Texas).

BHL1

The BHL1 insert corresponds to SEQ ID NO 5. Oligonucleotide pairs, ~~N4394/N4395, and N4396/N4397,~~N4394 (Seq ID NO. 54) /N4395 (Seq ID NO. 55), and N4396 (Seq ID NO. 56)/N4397 (Seq ID NO. 57), were annealed and ligated together to make a 202 base pair double stranded DNA molecule with overhangs compatible with *Rca* I and *Nhe* I restriction sites. PCR was performed on the annealed molecule using primers N5045 (Seq ID NO. 58) and N5046 (Seq ID NO. 59) to add a 5' *Spe* I site and 3' *Hind* III site. The PCR product was then restriction digested at those sites and ligated into pBluescript II KS+ at *Spe* I and *Hind* III sites.

The insert was then removed by restriction digestion with *Rca* I and *Hind* III and was ligated into the *Nco* I and *Hind* III sites of pET28a (Novagen) to form the BHL1 construct.

Oligonucleotide sequences (5' to 3'):

N4394 (Seq ID NO. 54)

1 CATGAAGCTG AAGACAGAGT GGCCGGAGTT GGTGGGGAAA TCGGTGGAGA

51 AAGCCAAGAA GGTGATCCTG AAGGACAAGC CAGAGGCGCA AATCATAGTT

101 CTGC

N4395 (Seq ID NO. 55)

1 CAACCGGCAG AACTATGATT TGCGCCTCTG GCTTGTCCTT CAGGATCACC

51 TTCTTGGCTT TCTCCACCGA TTTCCCCACC AACTCCGGCC ACTCTGTCTT

101 CAGCTT

N4396 (Seq ID NO. 56)

1 CGGTTGGTAC AAAGGTGACG AAGGAATATA AGATCGACCG CGTCAAGCTC

51 TTTGTGGATA AAAAGGACAA CATCGCGCAG GTCCCCAGGG TCGG

N4397 (Seq ID NO. 57)

1 CTAGCCGACC CTGGGGACCT GCGCGATGTT GTCCTTTTTA TCCACAAAGA

51 GCTTGACGCG GTCGATCTTA TATTCCTTCG TCACCTTTGT AC

N5045 (Seq ID NO. 58)

1 GTACTAGTCA TGAAGCTGAA GACAGA

N5046 (Seq ID NO. 59)

GAGAAGCTTG CTAGCCGACC CTGGGGAC

BHL2

The BHL2 construct insert corresponds to SEQ ID NO 7. An overlap PCR strategy was used to make the BHL2 construct. PWO polymerase from Boehringer-Mannheim was used for all PCR reactions. The primers were chosen to change 3 amino acids in the BHL1 active site loop region, and to create unique *Age* I and *Hind* III restriction sites flanking the active site loop, to facilitate loop replacement in future constructs. A unique *Rca* I site (compatible with *Nco* I) was lincluded at the 5' end, and a unique *Xho* I site was included at the 3' end. The overlap PCR was done as follows: PCR was done with primers N13561 ~~and N13564,~~(Seq. Id No. 60) and N13564 (Seq. Id No. 63), using the BHL1 construct as template. A separate PCR was done with primers N13563 ~~and N13562,~~(Seq. Id No. 62) and N13562 (Seq. Id No. 61) again using the BHL1 construct as template. The products from both reactions were gel purified and combined. Primer N13565 (Seq. Id No. 64), which overlapped regions on both of the PCR products, was then added and another PCR was done to generate the full-length insert. The resulting product was amplified by another PCR with primers N13561 (Seq. Id No. 60) and ~~and N13562.~~N13562 (Seq. Id No. 61). It was subsequently suspected that a deletion was present in N13562 (Seq. Id No. 61) that caused a frameshift near the 3' end of the PCR product. To avoid this frameshift problem, a final PCR reaction was done with primers N13562 (Seq. Id No. 61) and N13905 (Seq. Id ~~and N13905.~~No. 65). The final PCR product was digested with *Rca* I and *Xho* I, and then ligated into the *Nco* I and *Xho* I sites of pET 28b. Note: Some primers had 6-oligonucleotide extensions to improve restriction digestion efficiency.

Oligonucleotide sequences (5' TO 3'):

N13561 (Seq. Id No. 60)

1 TTTTTTTCATGAAGCTGAAGACA

N13562 (Seq. Id No. 61) (as ordered)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGA

N13563 (Seq. Id No. 62)

1 ATCGACAAGGTCAAGCTTTTTGTGGATAAAAAGGA

N13564 (Seq. Id No. 63)

1 CACCTTTGTACCAACCGGTAGAACTATGATTTGCGC

N13565 (Seq. Id No. 64)

1 GTTGGTACAAAGGTGGCGAAGGCCTATAAGATCGACAAGGTCAAG

N13905 (Seq. Id No. 65)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGACCTGCGCTA

BHL3

The BHL3 construct insert corresponds to SEQ ID NO 9. The BHL2 construct was digested with *Age* I and *Hin*d III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N14471 ~~and N14472,~~(Seq. Id No. 66) and N14472 (Seq. Id No. 67), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hin*d III restriction sites. The annealed product was ligated into the *Age* I and *Hin*d III sites of the digested BHL2 construct to yield the BHL3 construct.

Oligonucleotide sequences (5' to 3'):

N14471 (Seq. Id No. 66)

1 CCGGTTGGTACAAAGGTGGGTAAGCATTATAAGATCGACAAGGTCA

N14472 (Seq. Id No. 67)

AGCTTGACCTTGTCGATCTTATAATGCTTACCCACCTTTGTACCAA

BHL3N

The BHL3N construct insert corresponds to SEQ ID No 11. A PCR reaction was done with the BHL3 construct as template. The primers for this reaction were N13771 ~~and N13905.~~(Seq. Id No. 68) and N13905 (Seq. Id No. 65). The resulting PCR product was digested with *Rca* I and *Xho* I and ligated into the *Nco* I and *Xho* I sites of pET 28b to yield the BHL3N construct.

Oligonucleotide sequences (5' to 3'):

N13771 (Seq. Id No. 68)

1

TTTTTTTCATGAAGTCGGTGGAGAAGAAACCGAAGGGTGTGAAGACAGGTG

CGGGTGACAAGCATAAGCTGAAGACAGAGTG

N13905 (Seq. Id No. 65) (already provided in BHL2 description).

BHL4

The BHL4 construct insert DNA corresponds to SEQ ID NO 13. The BHL2 construct was digested with *Age* I and *Hind* III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N22098 ~~and N22099,~~(Seq. Id No. 69) and N22099 (Seq. Id No. 70), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hind* III restriction sites. The annealed product was ligated into the *Age* I and *Hind* III sites of the digested BHL2 construct to yield the BHL4 construct.

Oligonucleotide sequences (5' to 3'):

N22098 (Seq. Id No. 69)

CCGGTTGGTACAAAGGTGACGGGCGAATACAAGATCGACCGCGTCA

N22099 (Seq. Id No. 70)

AGCTTGACGCGGTCGATCTTGTATTCGCCCGTCACCTTTGTACCAA

BHL5

The BHL5 construct insert DNA corresponds to SEQ ID NO 15. This gene was synthesized by a commercial vendor, The Midland Certified Reagent Company (Midland,~~–~~Texas). The gene was supplied by Midland following digestion by *Nco* I and *Hind* III, and was ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL5 construct.

BHL6

The BHL6 construct insert DNA corresponds to SEQ ID NO 17. The BHL5 construct was digested with *Age* I and *Sal* I, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N23923 ~~and N23924,~~(Seq. Id No. 71) and N23924 (Seq Id. No 72), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Sal* I restriction sites. The annealed product was ligated into the *Age* I and *Sal* I sites of the digested BHL5 construct to yield the BHL6 construct.

Oligonucleotide sequences (5' to 3'):

N23923 (Seq. Id No. 71)

CCGGTGAATGGAAGATGGATCGCGTCCGCCTCTGGG

N23924 (Seq Id. No 72)

TCGACCCAGAGGCGGACGCGATCCATCTTCCATTCA

BHL8

The BHL8 construct insert DNA corresponds to SEQ ID No 19. A PCR reaction was done using the BHL6 construct as template. The primers for this reaction were N26671 ~~and N26672.~~(Seq ID. No 73) and N26672 (Seq ID. No 74). The resulting PCR product was digested with *Nco* I and *Hind* III and ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL8 construct.

Oligonucleotide sequences (5' to 3'):

N26671 (Seq ID. No 73)

TTTTTTCCATGGCTAAGATGAAGTGCACGTGGCCTGAGCTGGT

N26672 (Seq ID. No 74)

TTTTTTAAGCTTGGATCCCTAGCCGCACTTCGGAGTCTTGGCGA

The following experiments used truncated wild type CI-2.

Example 2 - Expression of BHL Proteins in *E. coli*, Purification, and Verification of Recombinant Protein Sequence

Expression in E. coli

BHL1, BHL2, BHL3, BHL3N, BHL4, BHL5, BHL6, BHL8, and the truncated wild-type CI-2 were expressed in *E. coli* using materials and methods from Novagen, Inc. The Novagen expression vector pET-28 was used (pET-28a for WT CI-2 and BHL1, and pET-28b for the other proteins). *Ecoli* strains BL21(DE-3) or BL21(DE-3)pLysS were used. Cultures were typically grown until an OD at 600 nm of 0.8 to 1.0, and then induced with 1 mM IPTG and grown another 2.5 to 5 hours before harvesting. Induction at an OD as low as 0.4 was also done successfully. Growth temperatures of 37 degrees centigrade and 30 degrees centigrade were both used successfully. The media used was 2xYT plus the appropriate antibiotic at the concentration recommended in the Novagen manual.

Exhibit B

Amendments to Specification, Pages 49 – 54, Clean

(i.e., substrates, products, inhibitors, activators), pH, ionic strength, and temperature will be controlled so as to obtain useful kinetic data and determine the presence of absence of a compound that binds or modulates protease inhibitor polypeptide activity. Methods of measuring enzyme kinetics is well known in the art. See, e.g., Segel, *Biochemical Calculations*, 2nd ed., John Wiley and Sons, New York (1976).

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

Example 1- Construction of Expression Cassettes

Vector construction was based upon the published WT CI-2A sequence information Williamson *et al,* Eur. J. Biochem 165: 99-106 (1987) and SEQ ID NO 1. Methods for obtaining full length or truncated wild-type CI-2 DNA include, but are not limited to PCR amplification, from a barley (or other plant) endosperm cDNA library using oligonucleotides derived from Seq. ID No 1 or from the published sequence *supra,* using probes derived from the same on a barley endosperm cDNA library, or using a set of overlapping oligonucleotides that encompass the gene, or having the gene synthesized by a commercial vendor such as The Midland Certified Regeant Company (Midland, Texas).

BHL1

The BHL1 insert corresponds to SEQ ID NO 5. Oligonucleotide pairs, N4394 (Seq ID NO. 54) /N4395 (Seq ID NO. 55), and N4396 (Seq ID NO. 56)/N4397 (Seq ID NO. 57), were annealed and ligated together to make a 202 base pair double stranded DNA molecule with overhangs compatible with *Rca* I and *Nhe* I restriction sites. PCR was performed on the annealed molecule using primers N5045 (Seq ID NO. 58) and N5046 (Seq ID NO. 59) to add a 5' *Spe* I site and 3' *Hind* III site. The PCR product was then restriction digested at those sites and ligated into pBluescript II KS+ at *Spe* I and *Hind* III sites. The insert was then removed

by restriction digestion with *Rca* I and *Hin*d III and was ligated into the *Nco* I and *Hin*d III sites of pET28a (Novagen) to form the BHL1 construct.

Oligonucleotide sequences (5' to 3'):

N4394 (Seq ID NO. 54)

1 CATGAAGCTG AAGACAGAGT GGCCGGAGTT GGTGGGGAAA TCGGTGGAGA

51 AAGCCAAGAA GGTGATCCTG AAGGACAAGC CAGAGGCGCA AATCATAGTT

101 CTGC

N4395 (Seq ID NO. 55)

1 CAACCGGCAG AACTATGATT TGCGCCTCTG GCTTGTCCTT CAGGATCACC

51 TTCTTGGCTT TCTCCACCGA TTTCCCCACC AACTCCGGCC ACTCTGTCTT

101 CAGCTT

N4396 (Seq ID NO. 56)

1 CGGTTGGTAC AAAGGTGACG AAGGAATATA AGATCGACCG CGTCAAGCTC

51 TTTGTGGATA AAAAGGACAA CATCGCGCAG GTCCCCAGGG TCGG

N4397 (Seq ID NO. 57)

1 CTAGCCGACC CTGGGGACCT GCGCGATGTT GTCCTTTTTA TCCACAAAGA

51 GCTTGACGCG GTCGATCTTA TATTCCTTCG TCACCTTTGT AC

N5045 (Seq ID NO. 58)

1 GTACTAGTCA TGAAGCTGAA GACAGA

N5046 (Seq ID NO. 59)

GAGAAGCTTG CTAGCCGACC CTGGGGAC

BHL2

The BHL2 construct insert corresponds to SEQ ID NO 7. An overlap PCR strategy was used to make the BHL2 construct. PWO polymerase from

Boehringer-Mannheim was used for all PCR reactions. The primers were chosen to change 3 amino acids in the BHL1 active site loop region, and to create unique *Age* I and *Hind* III restriction sites flanking the active site loop, to facilitate loop replacement in future constructs. A unique *Rca* I site (compatible with *Nco* I) was lincluded at the 5' end, and a unique *Xho* I site was included at the 3' end. The overlap PCR was done as follows: PCR was done with primers N13561 (Seq. Id No. 60) and N13564 (Seq. Id No. 63), using the BHL1 construct as template. A separate PCR was done with primers N13563 (Seq. Id No. 62) and N13562 (Seq. Id No. 61) again using the BHL1 construct as template. The products from both reactions were gel purified and combined. Primer N13565 (Seq. Id No. 64), which overlapped regions on both of the PCR products, was then added and another PCR was done to generate the full-length insert. The resulting product was amplified by another PCR with primers N13561 (Seq. Id No. 60) and N13562 (Seq. Id No. 61). It was subsequently suspected that a deletion was present in N13562 (Seq. Id No. 61) that caused a frameshift near the 3' end of the PCR product. To avoid this frameshift problem, a final PCR reaction was done with primers N13562 (Seq. Id No. 61) and N13905 (Seq. Id No. 65). The final PCR product was digested with *Rca* I and *Xho* I, and then ligated into the *Nco* I and *Xho* I sites of pET 28b. Note: Some primers had 6-oligonucleotide extensions to improve restriction digestion efficiency.

Oligonucleotide sequences (5' TO 3'):

N13561 (Seq. Id No. 60)

1 TTTTTTTCATGAAGCTGAAGACA

N13562 (Seq. Id No. 61) (as ordered)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGA

N13563 (Seq. Id No. 62)

1 ATCGACAAGGTCAAGCTTTTTGTGGATAAAAAGGA

N13564 (Seq. Id No. 63)

1 CACCTTTGTACCAACCGGTAGAACTATGATTTGCGC

N13565 (Seq. Id No. 64)

1 GTTGGTACAAAGGTGGCGAAGGCCTATAAGATCGACAAGGTCAAG

N13905 (Seq. Id No. 65)

1 TTTTTTCTCGAGGCTAGCCGACCCTGGGGACCTGCGCTA

BHL3

The BHL3 construct insert corresponds to SEQ ID NO 9. The BHL2 construct was digested with *Age* I and *Hind* III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N14471 (Seq. Id No. 66) and N14472 (Seq. Id No. 67), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hind* III restriction sites. The annealed product was ligated into the *Age* I and *Hind* III sites of the digested BHL2 construct to yield the BHL3 construct.

Oligonucleotide sequences (5' to 3'):

N14471 (Seq. Id No. 66)

1 CCGGTTGGTACAAAGGTGGGTAAGCATTATAAGATCGACAAGGTCA

N14472 (Seq. Id No. 67)

AGCTTGACCTTGTCGATCTTATAATGCTTACCCACCTTTGTACCAA

BHL3N

The BHL3N construct insert corresponds to SEQ ID No 11. A PCR reaction was done with the BHL3 construct as template. The primers for this reaction were N13771 (Seq. Id No. 68) and N13905 (Seq. Id No. 65). The resulting PCR product was digested with *Rca* I and *Xho* I and ligated into the *Nco* I and *Xho* I sites of pET 28b to yield the BHL3N construct.

Oligonucleotide sequences (5' to 3'):

N13771 (Seq. Id No. 68)

1

TTTTTTTCATGAAGTCGGTGGAGAAGAAACCGAAGGGTGTGAAGACAGGTG

CGGGTGACAAGCATAAGCTGAAGACAGAGTG

N13905 (Seq. Id No. 65) (already provided in BHL2 description).

BHL4

The BHL4 construct insert DNA corresponds to SEQ ID NO 13. The BHL2 construct was digested with *Age* I and *Hind* III, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N22098 (Seq. Id No. 69) and N22099 (Seq. Id No. 70), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Hind* III restriction sites. The annealed product was ligated into the *Age* I and *Hind* III sites of the digested BHL2 construct to yield the BHL4 construct.

Oligonucleotide sequences (5' to 3'):

N22098 (Seq. Id No. 69)

CCGGTTGGTACAAAGGTGACGGGCGAATACAAGATCGACCGCGTCA

N22099 (Seq. Id No. 70)

AGCTTGACGCGGTCGATCTTGTATTCGCCCGTCACCTTTGTACCAA

BHL5

The BHL5 construct insert DNA corresponds to SEQ ID NO 15. This gene was synthesized by a commercial vendor, The Midland Certified Reagent Company (Midland,Texas). The gene was supplied by Midland following digestion by *Nco* I and *Hind* III, and was ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL5 construct.

BHL6

The BHL6 construct insert DNA corresponds to SEQ ID NO 17. The BHL5 construct was digested with *Age* I and *Sal* I, and the region between these sites was removed by gel purification and discarded. Oligonucleotide pairs, N23923 (Seq. Id No. 71) and N23924 (Seq Id. No 72), were annealed to make a double stranded DNA molecule with overhangs compatible with *Age* I and *Sal* I restriction sites. The annealed product was ligated into the *Age* I and *Sal* I sites of the digested BHL5 construct to yield the BHL6 construct.

Oligonucleotide sequences (5' to 3'):

N23923 (Seq. Id No. 71)

CCGGTGAATGGAAGATGGATCGCGTCCGCCTCTGGG

N23924 (Seq Id. No 72)

TCGACCCAGAGGCGGACGCGATCCATCTTCCATTCA

BHL8

The BHL8 construct insert DNA corresponds to SEQ ID No 19. A PCR reaction was done using the BHL6 construct as template. The primers for this reaction were N26671 (Seq ID. No 73) and N26672 (Seq ID. No 74). The resulting PCR product was digested with *Nco* I and *Hind* III and ligated into the *Nco* I and *Hind* III sites of pET 28b to yield the BHL8 construct.

Oligonucleotide sequences (5' to 3'):

N26671 (Seq ID. No 73)

TTTTTTCCATGGCTAAGATGAAGTGCACGTGGCCTGAGCTGGT

N26672 (Seq ID. No 74)

TTTTTTAAGCTTGGATCCCTAGCCGCACTTCGGAGTCTTGGCGA

The following experiments used truncated wild type CI-2.

Example 2 - Expression of BHL Proteins in *E. coli*, Purification, and Verification of Recombinant Protein Sequence

Expression in E. coli

BHL1, BHL2, BHL3, BHL3N, BHL4, BHL5, BHL6, BHL8, and the truncated wild-type CI-2 were expressed in *E. coli* using materials and methods from Novagen, Inc. The Novagen expression vector pET-28 was used (pET-28a for WT CI-2 and BHL1, and pET-28b for the other proteins). *Ecoli* strains BL21(DE-3) or BL21(DE-3)pLysS were used. Cultures were typically grown until an OD at 600 nm of 0.8 to 1.0, and then induced with 1 mM IPTG and grown another 2.5 to 5 hours before harvesting. Induction at an OD as low as 0.4 was also done successfully. Growth temperatures of 37 degrees centigrade and 30 degrees centigrade were both used successfully. The media used was 2xYT plus the appropriate antibiotic at the concentration recommended in the Novagen manual.

Exhibit C

Amendments to Specification, Abstract, Page 76, Marked Version

The invention provides isolated nucleic acids and their encoded polypeptides that are involved in enhancing the essential amino acid content of a plant. The polypeptide may be derived from a protease inhibitor, and more specifically, a chymotrypsin inhibitor. Chymotrypsin inhibitors that may be modified for use in the invention are present in many plant species. ~~,including barley.~~ Barley (Hordeum vulgare) was initially used to obtain the chymotrypsin inhibitor modified for use in the present invention. Other plant species that may be used as a source for chymotrypsin inhibitor for use in the present invention include Zea Mays, Vicia faba, Cucurbita maxima, Canavalia lineata, Vigna angularis, Nicotiana tabacum, Nicotiana sylvestris, Sambucus nigra, Momordica charantia, Solanum tuberosum, Lycopersicon peruvianum, Lycopersicon esculentum, Amaranthus caudatus and Arabidopsis thalania. Optionally there is also a decrease in protease inhibitory activity of the polypeptide. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions. The present invention provides methods and compositions relating to increasing essential amino acid content of plants for feed.

Exhibit D

Marked Version of Amended Claims

Please amend the claims as follows:

9. (~~three~~(four times amended) A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and modified in order to have a composition selected from one of the following: at least~~15-35 mole % lysine, at least~~ 5-15 mole % methionine, at least 6-25 mole % threonine, and at least 4-9 mole % tryptophan; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.

12. (~~once~~(Twice amended) The polypeptide of Claim 10 wherein the protein exhibits reduced inhibitory activity against chymotrypsin, subtilisin or elastase when compared with the inhibitory activity against chymotrypsin, subtilisin or elastase exhibited by ~~wild-type CI-2.~~Seq. ID No. 4.

14. (~~once~~(Twice amended) The polypeptide of claim 10, further comprising one of the following pairs of substitutions: T22C and V82C; or E23C and R81C.

18. (~~twice~~(three times amended) The polypeptide of Claim 15 wherein the amino-terminal extension comprises at least one to~~about~~ eighteen additional residues corresponding to amino acid residues 1 to 18 of Seq. ID No. 2 or 12.

19. (~~twice~~(three times amended) A polypeptide comprising Seq. ID No. 2 modified to contain two or more ~~of the following~~modifications, said two or more modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:

H18A, H18I, H18L, H18V, H18M, N19K, N19T, L20M, L20I, L20V, E23T, E23K, S31T, S32K, E34K, E34T, V38M, V38I, V38L, L40M, L40I, L40V, Q41K, Q41T, Q47K, Q47T, I49M, I49I, I49L, I49V, I56K, I56T, M59G, R62K, R62T, I63M, I63L, I63V, R65K, R65T, R67K, R67T, F69W, L73K, L73T, N75K, N75T, Q78K, Q78T, V79T, V79K, R81K, and R81T; and

further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

21. (~~twice~~(three times amended) A polypeptide comprising Seq. ID No. 2 modified to contain two or more ~~of the following~~modifications, said two or more modifications corresponding to positions in Seq. ID No. 2 selected from the group consisting of:

H18A, H18M, N19K, L20M, T22C, E23T, E23C, S31T, E34K, V38M, L40M, Q41K, Q47K, I49M, I56K, M59G, R62K, I63M, R65K, R67K, F69W, L73K, N75K, Q78K, V79T, R81K, R81C, and V82C; and

further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

28. (~~twice~~(three times amended) A polypeptide comprising Seq. ID No. 2 modified to contain three or more modifications, said three or more modifications comprising non-native essential amino acids replacing native amino acids at positions corresponding to Seq. ID No. 2 and selected from the group consisting of positions

1, 8, 11, 17, 18, 19, 20, 22, 23, 31, 32, 34, 38, 40, 41, 45, 47, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 67, 69, 73, 74, 75, 76, 77, 78, 79, 81 and 82; and excluding V and W at position 56; K, V and W at position 58; W, V and K at position 59; T, I and K at position 60; V and W at position 61 and V and F at position 62; and

further provided that the polypeptide is a nutritional supplement and has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

29. (~~twice~~(three times amended) An isolated polypeptide comprising Seq. ID No. 6, 8, 10, 12, 14, 16, 18, 20 or ~~conservatively substituted variants thereof~~.
a conservative substitution thereof, wherein said polypeptide or conservative substitution thereof has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters

30. (~~twice amended) A~~(three times amended) An isolated polypeptide comprising at least twenty three contiguous amino acids of Seq. ID Nos. 6, 8, 10, 12, 14, 16, 18 or ~~20.~~
20, wherein said polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 2, wherein the percent identity is determined by Blast 2.0 using default parameters.

31. (~~twice~~(three times amended) An isolated polypeptide with more than 79% sequence identity to the polypeptide of Seq. ID No. 20, wherein the percent sequence identity is determined by GAP analysis using Gap Weight of 12 and Length Weight of 4.

59. (~~twice~~(three times amended) An isolated polypeptide with at least 60% sequence identity to the polypeptide of Seq. ID No. 2 comprising greater than fifty amino acids in length and comprising more than ~~seven~~ten lysine amino acid residues.

61. (~~once~~(twice amended)~~A polypeptide selected from the group consisting of~~:
~~(a) a~~An isolated polypeptide comprising Seq. ID Nos. 6, 8, 10, 12, 14, 16, 18 or ~~20; and~~ 20.

~~(b) a polypeptide comprising any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein said polypeptide has been modified to contain more than four~~63. (once amended) The

polypeptide of claim 72, wherein the non-native essential amino acidsat ~~positions corresponding to Seq. ID No. 2 positions 19-53 and 70-83, and wherein saidmodified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 or 20, the percent sequence identity determined by Blast 2.0 using default parameters~~.

~~63.~~ ~~(no change) The polypeptide of claim 61, wherein the essential~~ amino acid ~~is~~are isoleucine, lysine, tryptophan, methionine, threonine, or mixtures thereof.

64. ~~(no change)~~(once amended) The polypeptide of claim ~~61~~,72, wherein the non-native essential amino ~~acid is~~acids are lysine.

~~65.~~ ~~(no change)~~65.(once amended) The polypeptide of claim ~~61~~,72, further comprising an amino terminal extension.

69. ~~(once~~(twice amended) The polypeptide of claim ~~68~~,72, wherein the non-native di-sulfide bond is with a non-native cysteine~~is~~ at one or more positions corresponding to Seq. ID No. 24 positions 23, 81, 22, 82, 53 or 70.

70. ~~(no change)~~(once amended) The polypeptide of claim ~~61~~,72, further comprising at least two non-native cysteines.

72. ~~(once~~(twice amended) A polypeptide comprising~~any one of~~ Seq. ID ~~Nos.~~ 2,No. 4,6, ~~8, 10~~, 12, 14, ~~16~~, 18 ~~or 20~~, wherein said polypeptide has been modified to contain at least one non-native disulfide bond and more than four non-native essential~~amino acids in positions corresponding to Seq. ID No. 2 positions 19-83~~,amino acids, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 ~~or 20~~, the percent sequence identity determined by Blast 2.0 using default parameters.

76. (~~once~~(twice amended) A polypeptide comprising ~~any one of Seq. ID Nos. 2, 4, 6, 8, 10, 12, 14, 16, 18 or 20, wherein~~ the Seq. ID No. 4, wherein said polypeptide has been modified to contain ~~more than seven~~at least eleven non-native essential amino acids ~~in positions corresponding to Seq. ID No. 2 positions 19-53 and 70-83~~, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 4 ~~or 20~~, the percent sequence identity determined by Blast 2.0 using default parameters.

~~79.~~ (~~once~~78. (twice amended) A polypeptide having at least 80% ~~60%~~ sequence identity to the polypeptide of Seq. ID No. 2 4 and modified to contain a non-native disulfide bond.

~~60%~~79. (twice amended) A polypeptide having at least 80% identity to the polypeptide of Seq. ID No. 6 and modified to contain a non-native disulfide bond.

80. (~~once~~(twice amended) The polypeptide of claim 79, having at least ~~70%~~90% sequence identity to the polypeptide of Seq. ID No. 6.

81. (~~once~~(twice amended) A polypeptide having at least ~~60%~~80% sequence identity to the polypeptide of Seq. ID No. 8 and modified to contain a non-native disulfide bond.

82. (once(twice amended) The polypeptide of claim 81, having at least 70%90% sequence identity to the polypeptide of Seq. ID No. 8.

83. (~~once~~(twice amended) A polypeptide having at least ~~60%~~80% sequence identity to the polypeptide of Seq. ID No. 10 and modified to comprise a non-native disulfide bond.

84. (~~once~~(twice amended) The polypeptide of claim 83, having at least 70%90% sequence identity to the polypeptide of Seq. ID No. 10.

87. (~~once~~(twice amended) A polypeptide comprising any one of Seq. ID Nos. 24, 26, 28, 30, 32 or 35-53, wherein the polypeptide is modified to have a non-native disulfide bond and more than seven non-native essential amino acid ~~residues~~.
residues, and wherein said modified polypeptide has at least 30% sequence identity to the polypeptide of Seq. ID No. 2 as determined by Blast 2.0 using default parameters.

98. (new) A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and modified in order to have a composition of at least 15.00-35.00 mole % lysine; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.

99. (new) A polypeptide with at least 30% sequence identity to the polypeptide of Seq. ID No. 2 and comprising greater than fifty amino acids in length and modified in order to have a composition of at least 15.00-25.00 mole % lysine; wherein the % sequence identity is based on the entire sequence and is determined by BLAST 2.0 using default parameters.